PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/505, 31/335, 31/70

A1

(11) International Publication Number: WO 95/08994

(43) International Publication Date: 6 April 1995 (06.04.95)

(21) International Application Number: PCT/US94/09949

(22) International Filing Date: 2 September 1994 (02.09.94)

(30) Priority Data:
08/128,843 29 September 1993 (29.09.93) US

(71) Applicant: INDIANA UNIVERSITY FOUNDATION [US/US]; Showalter House, P.O. Box 500, Bloomington, IN 47404 (US).

(72) Inventors: WEBER, George; 702 Barnhill Drive, Indianapolis, IN 46202 (US). LOOK, Katherine, Y.; 550 North University Boulevard, Indianapolis, IN 46202 (US).

(74) Agents: FISH, Robert, D. et al.; Lyon & Lyon, 611 West Sixth Street, 34th floor, Los Angeles, CA 90017 (US).

(81) Designated States: AU, BR, CA, JP, KR, NZ, RU, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: ANTINEOPLASTIC COMBINATION OF TAXOL AND TIAZOFURIN

(57) Abstract

A method of treating neoplastic cells using a combination of taxol and tiazofurin. Preferably, the taxol is administered in a dosage of approximately 110 mg/m^2 to approximately 250 mg/m^2 on a single day, followed by 31 days of rest, and the tiazofurin is administered in a dosage of approximately 1,800 to 4,400 mg/m^2 over a period of about ten days, followed by 21 days of rest, and the taxol and tiazofurin administration cycles are in phase with each other.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

DESCRIPTION

ANTINEOPLASTIC COMBINATION OF TAXOL AND TIAZOFURIN

I. Background of the Invention

A. Field of the Invention

The field of the present invention is the treatment of neoplastic cells utilizing chemotherapeutic agents.

B. Background Information

Control of cancer remains a much sought after goal. In fact, Lung cancer is a major therapeutic problem, with 168,000 new cases per year in the United States, 87% mortality, and a continuing rise of incidence in both men and women. With regard to pancreatic cancer, which is the fifth leading cause of cancer deaths in the United States, and which carries a 90% mortality rate, 28,000 new cases are diagnosed annually. Chemotherapy in this disease has so far been useful only as a palliative. It also is estimated that there will be 22,000 new cases of ovarian cancer in the United States in 1993. Thus, there is a need for new therapies to treat and control cancers, including especially lung, pancreatic and ovarian cancers.

Neoplastic cells tend to grow and divide faster than ordinary cells, and several anti-cancer drugs have been developed which interfere with cell division. Two such drugs, taxol (NSC 125975) and tiazofurin (NSC 286193), interfere with the formation of microtubules, structural elements employed in the formation of the mitotic spindle. Microtubules are produced through polymerization of alpha-tubulin and beta-tubulin, under the influence of GTP (guanosine triphosphate) and microtubulin-associated proteins.

As shown in Figure 1, taxol and tiazofurin interfere with mitotic spindle formation at two distinct sites. Taxol exerts its anti-cancer action by binding to the

microtubule and promoting precocious microtubule assembly. It does not inhibit the binding or the hydrolysis of GTP. (Manfredi, J.J. and Horwitz, S.B., "Taxol: An antimitotic agent with a new mechanism of action", Pharmac. Ther. 25:83, 1984; Schiff, P. B. and Horwitz, S. B., "Taxol assembles tubulin in the absence of exogenous guanosine 5-triphosphate or microtubule-associated proteins", Biochemistry, 20:3247, 1981).

Tiazofurin exerts its anti-cancer action through conversion to an active metabolite, TAD (thiazole-4-carboxamide adenine dinucleotide), which then inhibits IMPDH (inosine 5'phosphate dehydrogenase), the rate limiting enzyme of de novo GTP synthesis. Weber G., "Biochemical strategy of cancer cells and the design of chemotherapy," H.A. Clowes Memorial Lecture, in Cancer Res 43:3466-3492, 1983; Weber, G., "IMP Dehydrogenase and GTP as Targets in Human Leukemia Treatment" Purine and Pyrimidine Metabolism in Man VII, pp. 287-292 (Plenum Press, NY 1991; Cooney, D., et al, "The conversion of 2-beta-D-ribofuanosylthiazole-4-carboxamide to an analog of NAD with potent IMP-dehydrogenase inhibitory properties", Biochem. Pharmacol., 31:2133-2136, 1982). Look, et al. found that the addition of 0.5 micromolar TAD for 10 minutes to extracts of ovarian carcinoma led to 81% inhibitory effect of IMPDH activity. (Look, K. Y., et al, "Inhibition by tiazofurin of inosine 5'phosphate dehydrogenase (IMPDH) activity in extracts of ovarian carcinoma", Gynecol. Oncol., 47:66-70, 1992). Micha et al demonstrated an inhibitory effect of tiazofurin on growth of xenographs of human epithelial carcinoma in a mouse subrenal capsule assay. (Micha J.P., et al, "Action of 2-beta-D-ribofuanosylthiazole-4-carboxamide (tiazofurin) against untreated human ovarian cancers in the murine xenograph assay", Gynecol. Oncol., 21:351-355, 1985).

Clinical development of taxol as an anti-cancer agent in humans progressed slowly because of an initially limited drug supply and because of taxol's poor solubility

in aqueous solution. However, more recently taxol has been recognized as an important and highly promising drug. In a recent review, Chabner noted Phase I and Phase II investigations in taxol revealed clear indications of anti-tumor activity in otherwise refractory solid tumors. (Chabner, B. A., "Taxol", in V.T. De Vita Jr, S. Hellman, S. A. Rosenberg (eds.), "PPO Updates 5", Cancer Principles & Practice of Oncology, 3rd Edition, pp. 1-10, 1984).

Clinical development of tiazofurin as an anti-cancer agent also proceeded slowly. In the early clinical studies, the drug was given as a 10 minute bolus or as a continuous intravenous infusion, each of which caused various toxicities. As a result, low and ineffective dose schedules were used in patients with solid tumors in Phase I trials. In more recent clinical investigations, tiazofurin was given in effective doses as a daily one hour infusion. In these investigations, good therapeutic results were obtained in end stage leukemic patients, particularly in patients in blast crisis with chronic granulocytic leukemia, where a 77% response rate was seen. (Tricot, B. et al. "Tiazofurin: Biological Effects and Clinical Uses," Int'l J. Cell Cloning 8:161, 1990; Weber, G. et al. 1991 supra). This compares with a 25-50% response in such patients treated with conventional therapy.

The effectiveness of tiazofurin can be enhanced by the synergistic effect of allopurinol, (1,5-Dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one). Allopurinol decreases xanthine oxidase activity, which increases plasma hypoxanthine concentration, which in turn competitively inhibits the activity of GPRT and further inhibits the synthesis of GTP. (Weber, G., 1991 supra; Weber, G., et al, "Enzyme-pattern-targeted chemotherapy with tiazofurin and allopurinol in human leukemia", Adv. in Enzyme Regul., 27:405-433, 1988).

II. Summary of the Invention

The present invention is directed to treating neoplastic disease, solid tumors, and leukemia, using a combination of taxol and tiazofurin. Accordingly, it is an object of the present invention to provide a combination drug treatment for neoplastic disease. Other and further objects and advantages will appear hereinafter.

III. Detailed Description of the Preferred Embodiment

As used herein, taxol refers to 5β20-epoxy-1,2α,4,7β, 10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine, and pharmaceutically acceptable salts thereof (NSC 125975). As used herein, tiazofurin refers to 2-β-D-ribofuranosylthiazole-4-carboxamide, and pharmaceutically acceptable salts thereof (NSC 286193).

A. Preparation and Administration

Taxol is commercially available, and is administered at the rate of between approximately 110 mg/m^2 to approximately 250 mg/m^2 in a three hour infusion, once every 31 days. The starting dose of taxol is preferably 175 mg/m^2.

Tiazofurin may be prepared as described in U.S. Patent No. 4,680,285 or U.S. Patent No. 4,451,648, which are incorporated herein by reference. Tiazofurin also may be obtained from the National Cancer Institute as a sterile drug for injection in clear vials. Each vial contains 1 gram prepared as a white lyophilized powder with sodium hydroxide to adjust the pH. A tiazofurin solution is reconstituted by adding 4.6 ml of sterile water for injection USP the contents of each vial. One ml of the resulting solution will then contain 200 mg of tiazofurin with a pH of 6-8. The reconstituted solution is further diluted in 500 ml of 5% dextrose solutions, USP, or 0.9% Sodium Chloride solution, USP.

Tiazofurin is preferably administered by one-hour infusion as described in Jayaram, H. et al., "Clinical Pharmacoanalytic Study of Tiazofurin Administered as a One Hour Infusion", Int. J. Cancer, 51(2):182-188, 1992), incorporated herein by reference. In such infusions, tiazofurin is dissolved or suspended in a physiologically compatible solution, in a concentration of at least 0.1% by weight. More preferably, tiazofurin would be present in a concentration of about 10% to about 90% by weight.

Before a patient receives tiazofurin, baseline IMPDH activity is determined. In patients with ascites, the ascites is removed by paracentesis, and at least 50,000,000 cells are assayed for IMPDH activity. Only those patients whose baseline IMPDH is elevated, suggesting that the drug may have utility in view of its mechanism of action, receive tiazofurin. In such patients, tiazofurin is reconstituted and diluted as described above and is administered by infusion pump over one hour at a dose of 4,400 mg/m^2 per day for the first two days. If the IMPDH activity after two days is higher than 10% of baseline, the tiazofurin treatment is discontinued. If the IMPDH activity after two days is less than 10% of baseline, the patient is maintained on tiazofurin at a dose of 2,200 mg/m^2 per day, given as a one hour infusion for a total of 8 days. Between each cycle there is a 21 day rest, such that an entire treatment cycle is 31 days.

In subsequent cycles, if patients present with persistent ascites, the ascites is resampled. If, however, the ascites has resolved or become cytologically negative, and there is no other evidence of disease progression, the patient is continued on therapy as this would suggest at least a partial response.

On days that both taxol and tiazofurin are given, tiazofurin is preferably administered in the morning, and taxol is preferably administered in the evening. During the course of combined taxol and tiazofurin treatment as described herein, the patient's hematology is carefully

monitored. In the event of excessive toxicity, administration of one or both of taxol and tiazofurin is reduced or discontinued. In the event of severe anemia (hemoglobin concentration of less than 8 gm/100ml), taxol should be reduced to a dosage of about 110 mg/m^2. In the event severe leukopenia (less than 1000 absolute granulocyte count) tiazofurin should be reduced to a dosage of about 1800 mg/m^2.

The approximately eight day maintenance dose of tiazofurin depends on how well tiazofurin is tolerated by an individual patient. If tiazofurin appears to be well tolerated, the maintenance dosage is increased to 3,300 mg/m^2 from 2,200 mg/m^2. If tiazofurin appears to be poorly tolerated, the maintenance dosage is decreased to 1,800 mg/m^2 from 2,200 mg/m^2.

Allopurinol is preferably, but not necessarily used in conjunction with tiazofurin treatment. Allopurinol is commercially available in 100 mg tablets. During the tiazofurin treatment, the patient may receive 100 mg by month every four hours in order to attain plasma hypoxanthine levels of 40-80 micromoles. Should the allopurinol starting dose fail to achieve a hypoxanthine concentration of abut 40 μm, the allopurinol dose can be increased to a maximum of 100 mg by month every three hours. During the treatment, the amount of oral and intravenous fluids are limited to less than 3 liters per day to maintain plasma hypoxanthine levels, but at least 2 liters per day to minimize the risk of hypoxanthine renal stones.

The synergistic activity of taxol and tiazofurin in attacking microtubular synthetic processes of the mitotic spindle has been tested in human ovarian, pancreatic and lung carcinoma cells, and in rat hepatoma 3924A cells. As described below, taxol and tiazofurin proved synergistic in all four cell lines tested ($p > 0.05$). These results indicate that taxol and tiazofurin should also have synergistic effect in the clinical treatment of human

solid tumors, including tumors of the ovary, pancreas, lung and liver. Such improved effectiveness should permit dose reductions which result in lower toxicity, decreased emergence of resistant clones, and more rational modulation of chemotherapy schedules.

IV. Experimental Data on Cell Lines

The synergistic inhibitory action of taxol and tiazofurin on cell proliferation was studied in vitro. The investigations focused primarily on human cell lines originally derived from solid tumors of the ovary, pancreas, and lung.

A. Cell Lines Studied

The cell lines studied were human ovarian carcinoma OVCAR-5, human pancreatic carcinoma PANC-1, human non-small cell adenosquamous carcinoma H-235 cells and rat hepatoma 3924A cells. All cells were maintained in RPMI-1640 medium, supplemented with 10% FBS (GIBCO, Grand Island, NY), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were incubated in 5% CO_2 with 95% humidified air at 37°C. For subcultures, cells were dispersed with 0.25% trypsin containing 1 Mm EDTA at 37°C for 10 min, then centrifuged, suspended in fresh medium and seeded in culture flasks. Exponentially growing cells were seeded in 24-well plates in triplicate at a density of 2 x 10^4 cells/ml/well and drugs were added alone or simultaneously 6 hr later. After 3 days (OVCAR-5, 3924A cells) or 4 days (PANC-1, H-125 cells) of drug exposure, cells were harvested and counted in a Coulter counter.

B. Drugs Tested

Taxol (10mM) was prepared in DMSO (dimethyl sulfoxide) and diluted in PBS (phosphate balanced solution) for a 25 μM stock solution and from this solution aliquots were dissolved in RPMI-1640 medium. The highest concentration of taxol (0.025 μM) contained 0.0019% DMSO which had no

effect on the cells. Tiazofurin was dissolved in PBS. Stock solutions were stored at -20°C.

C. Evaluation of Drug Action

Means ± SE of cells in triplicate samples were tabulated and percent inhibition was calculated and compared with control. The Chou-Talalay method (Chou and Talalay, 1984) was used to determine the nature of the interaction of the two drugs. In the tables below, Fa denotes the fraction affected, and CI denotes the combination index. CI of less than 1 indicates synergism, values greater than 1 denote antagonism, and values equal to 1 reveal summation (additivity) (Chou and Talalay, 1984).

D. Results

Table I summarizes the effect of taxol and tiazofurin in the cell lines tested. The doubling times of OVCAR-5, PANC-1, H-125, and 3924A cells were 15, 36, 27, and 15 h, respectively. The IC_{50} values reveal the marked differences in the activities of taxol and tiazofurin (Fig. 2). Taxol is effective in nanomolar concentrations whereas tiazofurin requires concentrations 2 to 3 orders of higher magnitude (micromolar concentrations) to be effective. In these studies only minor variations were observed over a period of 1 year in the doubling times and IC_{50} values.

Table II summarizes the effect of taxol and tiazofurin in human ovarian carcinoma cells. Taxol (2 to 25 nM) stopped cell growth in 4 to 25% of the OVCAR-5 cells. Tiazofurin (5 to 15 μM) inhibited cell growth in 25 to 67% of the cells. The two drugs were synergistic in the concentrations tested as shown by the C.I. of 0.40 to .90. The most effective combination, however, included 25 nM taxol with 15 μM tiazofurin, (1:600 ratio), which inhibited 84% of cell growth

Table III summarizes the synergistic activity of taxol and tiazofurin in pancreatic cancer cells. Taxol (0.4 to 10 nM) stopped proliferation in 2 to 68% of the PANC-1 cells. Tiazofurin in concentrations of 0.1 through 10 μM inhibited 4 to 83% of cell proliferation. In the various combinations tested, synergism was observed against PANC-1 cells. In the best combination, 10 nM taxol and 10 μM tiazofurin yielded 96% inhibition of cell proliferation. A 10-fold increase (1 to 10 μM) in tiazofurin concentration yielded little anti-proliferative advantage. Hence, with respect to PANC-1 cells, the combination of 10 nM taxol and 1 μM tiazofurin, (1:100 ratio), was the most effective combination tested.

Table IV summarizes the synergistic activity of taxol and tiazofurin in human lung cancer cells. Taxol (0.4 to 10 nM) resulted in 12 to 42% inhibition of H-125 cell proliferation. Tiazofurin (0.1 to 10 μM) inhibited growth of 21 to 75% of the cells. The two drugs were synergistic in the concentrations tested with best results obtained with 10 nM taxol and 10 μM tiazofurin (1:1000 ratio), yielding 89% inhibition of cell proliferation. A 5-fold increase in taxol concentration to 10 nM in the presence of 10 μM tiazofurin improved results, with 89% inhibition of cell proliferation.

With respect to the synergistic action of taxol and tiazofurin in rat hepatoma 3924A cells, taxol (2 to 25 nM) inhibited 7 to 18% of cells and tiazofurin (1 to 10 μM) inhibited 15 to 62% of cells. In all combinations tested, taxol and tiazofurin were synergistic (not shown). The best combination appeared to be 25 nM taxol and 10 μM tiazofurin (1:400 ratio) which provided 81% inhibition of cell proliferation.

Thus, a method of treating neoplastic cells with a combination of taxol and tiazofurin has been disclosed. While specific embodiments and applications of this invention have been shown and described, it would be apparent to those skilled in the art that many more

modifications are possible without departing from the inventive concepts herein. The invention, therefore, is not to be restricted except in the spirit of the appended claims.

Claims

1. A method of treating neoplastic cells in a warm-blooded patient comprising the administration of a combination of taxol and tiazofurin.

2. The method of claim 1 further comprising the administration of allopurinol.

3. A method of treating neoplastic disease in a warm-blooded patient comprising:

administering a first pharmaceutical composition containing at least one of 5β20-epoxy-1,2α,4,7β, 10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine and a pharmaceutically acceptable salt thereof; and

administering a second pharmaceutical composition containing at least one of 2-β-D-ribofuranosylthiazole-4-carboxamide and a pharmaceutically acceptable salt thereof.

4. The method of claim 3 in which the first pharmaceutical composition is administered in an approximately 31 day cycle, during which a patient receives a dosage of between about 110 mg/m^2 and about 250 mg/m^2 during a single day, followed by about 31 days of rest.

5. The method of claim 3 in which the second pharmaceutical composition is administered in an approximately 31 day cycle, during which a patient receives a dosage of between about 1800 mg/m^2 and about 4,400 mg/m^2 per day over a period of about two to about ten days, followed by about 21 days of rest.

6. The method of claim 3 further comprising administration of allopurinol to attain plasma hypoxanthine serum levels of about 40-80 micromolar.

7. A method of treating neoplastic disease in a warm-blooded patient comprising:

administering a therapeutically effective dosage of a first pharmaceutical composition containing at least one of 5β20-epoxy-1,2α,4,7β, 10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine and a pharmaceutically acceptable salt thereof, said first composition administered at the rate of between approximately 110 mg/m^2 to approximately 250 mg^2 in an approximately three hour infusion.

determining the patient's baseline IMPDH level;

administering a therapeutically effective dosage of a second pharmaceutical composition containing at least one of 2-β-D-ribofuranosylthiazole-4-carboxamide and a pharmaceutically acceptable salt thereof, said second composition administered by infusion pump over approximately one hour at a dose of 4,400 mg/m^2 per day for approximately two days;

making a subsequent determination of the patient's IMPDH level;

comparing said subsequent IMPDH level with said baseline IMPDH activity; and

continuing administration of said second composition for up to an additional approximately eight days if said subsequent IMPDH level is less than approximately 10% of the baseline IMPDH level.

8. The method of claim 7, further comprising administration of approximately 100 mg of allopurinol by mouth approximately every three to four hours to attain plasma hypoxanthine serum levels of about 40-80 micromolar.

9. The method of claim 7 wherein the dosage of said first composition is reduced to a minimum of about 110 mg/m^2 in response to severe anemia.

10. The method of claim 7 wherein the dosage of said second composition is reduced to a minimum of about 1800 mg/m^2 in response to severe leucopenia.

11. The method of claim 7 wherein said first composition is administered in cyclical manner, in which each of said three hour infusions is followed by approximately 31 days of rest.

12. The method of claim 7 wherein said second composition is administered in an approximately 31 day cycle, in which the patient receives up to about ten consecutive days of said administration of said second composition followed by about 21 days of rest.

13. The method of claim 7 wherein both of said first and second compositions are administered in a cycle having a period of approximately 31 days.

14. The method of claim 13 wherein said first composition is administered in the evening and said second composition is administered in the morning on the first day of each of said cycles.

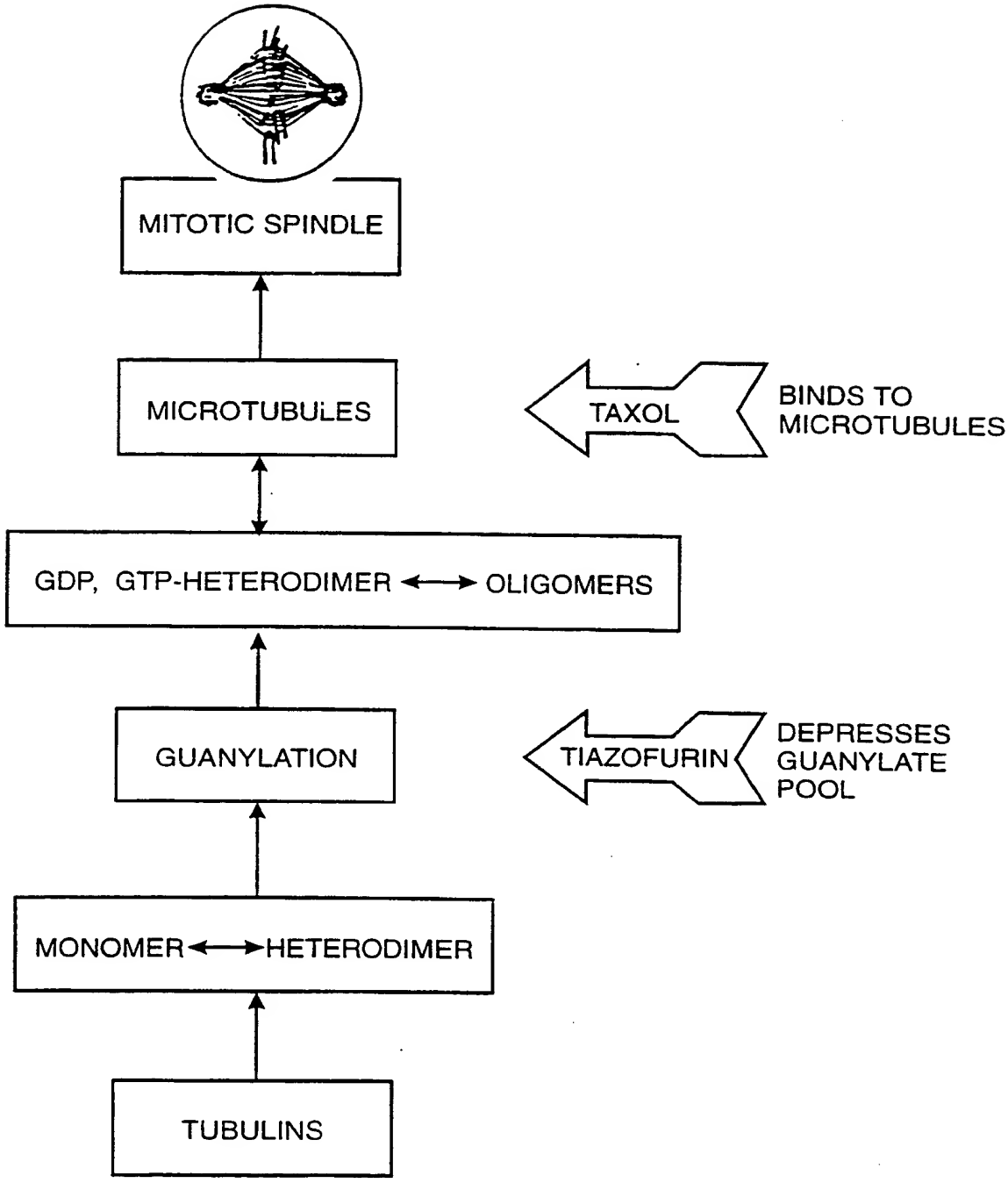

FIG. 1

TABLE I
Effect of taxol and tiazofurin on IC_{50} in culture

Cell Line	Origin	Histology	Doubling time	IC_{50}: μM	
			h	Taxol	Tiazofurin
OVCAR-5	Human	Ovarian carcinoma (adenocarcinoma)	15	0.05	8.3
PANC-1	Human	Pancreatic carcinoma (epitheloid carcinoma)	36	0.06	2.3
H-125	Human	Lung carcinoma (adenosquamous carcinoma)	27	0.03	1.8
3924A	Rat	Hepatoma (hepatocellular carcinoma)	15	0.04	6.9

TABLE II
Synergistic action of taxol and tiazofurin in human OVCAR-5 cells

Taxol (μM)	Tiazofurin (μM)	Fa	C.I.
0.002	—	0.04	—
0.010	—	0.12	—
0.025	—	0.25	—
—	5	0.25	—
—	10	0.45	—
—	15	0.67	—
0.002	5	0.37	0.74
0.002	10	0.57	0.84
0.010	10	0.61	0.83
0.010	15	0.71	0.90
0.025	5	0.64	0.50
0.025	10	0.79	0.49
0.025	15	0.84	0.57

TABLE III
Synergistic action of taxol and tiazofurin in human PANC-1 cells

Taxol (μM)	Tiazofurin (μM)	Fa	C.I.
0.0004	—	0.02	—
0.002	—	0.14	—
0.010	—	0.68	—
—	0.1	0.04	—
—	1	0.33	—
—	5	0.65	—
—	10	0.83	—
0.0004	0.1	0.09	0.89
0.0004	5	0.73	0.88
0.002	10	0.86	0.88
0.010	0.1	0.74	0.80
0.010	1	0.94	0.27
0.010	5	0.94	0.41
0.010	10	0.96	0.40

TABLE IV
Synergistic action of taxol and tiazofurin in human lung carcinoma H-125 cells

Taxol (μM)	Tiazofurin (μM)	Fa	C.I.
0.0004	—	0.12	—
0.002	—	0.11	—
0.010	—	0.42	—
—	0.1	0.25	—
—	1	0.27	—
—	5	0.60	—
—	10	0.79	—
0.0004	0.1	0.25	0.63
0.0004	10	0.81	0.32
0.002	0.1	0.28	0.85
0.002	1	0.45	0.99
0.002	10	0.85	0.19
0.010	0.1	0.63	0.13
0.010	1	0.56	0.60
0.010	5	0.78	0.26
0.010	10	0.89	0.10

INTERNATIONAL SEARCH REPORT

Inte.. ..tional application No.
PCT/US94/09949

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/505, 31/335, 31/70
US CL :514/258, 449, 43

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/258, 449, 43

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	Proceedings for the American Association for Cancer Research, vol. 34, issued March 1993, Taniki et al, " Synergistic action of Taxol and Tiazofurin in Human Ovarian, Pancreatic and Lung Carcinoma Cells", see page 297.	1- 14
Y	Advances in Enzyme Regulation, vol. 27, issued 1988, Weber et al, "Enzyme Pattern - Targeted Chemotherapy with Tiazofurin and Allopurinol in Human Leukemia", pages 405-443, see 424-428.	2,6,8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 07 DECEMBER 1994	Date of mailing of the international search report 03 JAN 1995
Name and mailing address f the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEROME D. GOLDBERG Telephone N . (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*